Temporal Variation in Population Genetic Structure of a Riverine African Cichlid Fish

ERIKA CRISPO AND LAUREN J. CHAPMAN

From the Department of Biology, McGill University, 1205 Avenue Dr Penfield, Montréal, QC, Canada H3A 1B1 (Crispo and Chapman).

Address correspondence to Erika Crispo at the address above, or e-mail: erika.crispo@mail.mcgill.ca.

Population genetic structure in a riverine cichlid fish was recharacterized 2 years after patterns had been first described. We found that genetic structure changed, as evidenced by changes in $F_{ST}$ between years among sites, significant $F_{ST}$ between years “within” sites, and a significant proportion of the genetic variation partitioned between years. Most striking, signatures of isolation by distance were eradicated between years. Our study highlights that point-in-time estimates of population genetic structure might not be valid over longer time periods, particularly in systems exposed to strong seasonal or interannual variation in abiotic conditions.

Key words: dispersal, isolation by distance, floods, $F_{ST}$, gene flow, genetic divergence

Examining population structure and gene flow in nature is imperative for understanding a variety of phenomena, such as speciation (e.g., Bernatchez and Wilson 1998; Martin and McKay 2004; Barluengoa et al. 2006) and biogeographical processes (e.g., Johnson and Taylor 2004; Fraser and Bernatchez 2005; Kotlik et al. 2008), and for establishing conservation units and priorities (DeSalle and Amato 2004; Pearse and Crandall 2004). Population structure is usually estimated using neutral genetic markers sampled at one point in time. When populations are sampled more than once, sampling is often performed over periods of time on the magnitude of 25–60 years (e.g., Meldgaard et al. 2003; Favé and Turgeon 2008; Sonstebo et al. 2008). Genetic structure can, however, fluctuate on time scales as short as 1–2 years (e.g., Lacson and Morizot 1991; Congdon 1995; González-Wangüemert et al. 2007; Beneteteau et al. 2009), and thus studies are necessary for determining how often and under what conditions rapid changes in population genetic structure are expected to occur.

Events such as floods, droughts, and hurricanes could have profound and immediate effects on the population structure of aquatic organisms, either by physically moving individuals across a landscape or by changing the landscape so that dispersal corridors are altered, which might either prevent or enhance dispersal (e.g., Lacson and Morizot 1991; Huey et al. 2008; Jowers et al. 2008; Masei et al. 2008). Flooding in river drainages might influence variation in population structure in aquatic riverine organisms. For example, evidence for downstream-biased gene flow is common in rivers (e.g., Congdon 1995; Hernandez-Martich and Smith 1997; Barson et al. 2009), but it is unclear whether these patterns remain constant or vary temporally with changes in the environment.

The river systems of western Uganda are subject to biannual flooding that might influence the structure of fish populations (Figure 1). This region is characterized by a number of rivers, as well as connected swamps, inhabited by a variety of fish species including cichlids, catfishes, anabantoids, killifish, and barbs (Crispo E and Chapman LJ, personal observations). Some potential effects of flooding on riverine fishes might include increased gene flow, particularly in the downstream direction (e.g., Congdon 1995; Huey et al. 2008; Barson et al. 2009); declines in population sizes due to the washing out of fish or changes in abiotic or biotic conditions (e.g., Fausch et al. 2001; Grether et al. 2001); altered patterns of natural selection due to changes in abiotic or biotic conditions (e.g., Congdon 1995; Grether et al. 2001); or genetic drift (e.g., Congdon 1995; Barson et al. 2009). We thus used fish from western Uganda to test the prediction that population structure of riverine organisms can fluctuate on short time scales in areas afflicted by periodic flooding. We specifically examine genetic variability, inbreeding coefficients, estimated population sizes, genetic differentiation, the apportionment of genetic variation among sites and between years, and the proportion of shared alleles.

Materials and Methods

We reexamined population genetic structure of a widespread African cichlid fish (Pseudocrenilabrus multicolor victoriae) 2 years
after it had been previously characterized (Crispo and Chapman 2008). This species can reproduce at an age of approximately 6 months in the laboratory and can survive for more than 2 years (i.e., individuals can survive longer than the period between sampling; Crispo E and Chapman LJ, personal observations). River and swamp populations of this species are subject to divergent selection related to dissolved oxygen, conductivity, and turbidity, and potentially prey availability, predation pressure, and competition with other species (Crispo E and Chapman LJ, personal observations). At least oxygen and conductivity are known to vary seasonally, and this variation might be attributable to rainfall (e.g., Chapman 1995; Chapman and Liem 1995; Chapman et al. 2002, 2004). Based on a previous study of population structure using neutral genetic markers, ecological barriers to gene flow were not uncovered in this species (i.e., no increased genetic divergence between river and swamp environments relative to within them), but physical barriers due to geographical separation and distance (i.e., isolation by distance) were present (Crispo and Chapman 2008).

In May–June 2008, we collected fin tissue from approximately 30 adult fish at each of 6 sites in the Mpanga watershed in western Uganda (Bunoga, Bwera, Rwabakwata, Kahunge, Kamwenge, and Kanyantale; Figure 2) using procedures described in Crispo and Chapman (2008). One swamp site was sampled in 2008 that had not previously been sampled (Kanyantale; Figure 2). Two sites that had previously been sampled in this watershed (Kiagarura and Kantembwe) were not sampled in 2008 due to logistic constraints. Contemporary dispersal among all sites should be possible at least during the wet season. Large portions of papyrus swamps in Uganda can become dry during the dry seasons, but these swamps can expand by nearly 300% during the wet seasons (Chapman 1995; Chapman and Liem 1995). These observations suggest that the Mpanga River and adjacent swamp might have increased connectivity during the wet seasons. Indeed, seasonal decreases in oxygen concentration at river sites downstream from the swamp in our study area suggest that swamp water, containing decomposing debris, flushes into the river during rainy periods (Chapman et al. 2008; Crispo and Chapman 2008). Sampling was performed throughout the range of P. multicolor within this one watershed. That is, P. multicolor could not be caught in abundance at sites located in the upstream areas of the drainage after extensive sampling (Aliganyira E, Omeja P, Twinomugisha D, personal communications; Chapman LJ, personal observations), and a large waterfall is located downstream, blocking upstream dispersal from other sites. No other watersheds lie close to the Mpanga in the area above the waterfall, making dispersal among watersheds unlikely, even during flooding.

We extracted DNA using DNeasy tissue kits (Qiagen) and genotyped 10 tetranucleotide microsatellite loci following procedures described in Crispo et al. (2007). To make between-year comparisons possible, we used loci and laboratory procedures identical to those used for the 2006 samples. That is, the same equipment, primers, and polymerase chain reaction conditions were used, and the same technician performed the laboratory work and identified the allele sizes (Crispo and Chapman 2008). Two loci that had previously been genotyped (Ppun2 and Ppun12) were not genotyped for this study because heterozygote deficits had previously been found, suggesting the possible presence of null alleles—these loci were not used in any analysis here or in the previous study (Crispo and Chapman 2008).
To assess the suitability of the microsatellite markers, we tested for the possible presence of null alleles and the linkage of loci. To test for heterozygote deficits and linkage equilibrium (LE), we used Genepop on the Web (Raymond and Rousset 1995). Exact tests for Hardy–Weinberg equilibrium (HWE) (1-tailed tests for heterozygote deficits) were performed for each locus within each site and across all sites, and LE was tested for each pair of loci within each site and across all sites. We tested both HWE and LE using the Markov chain, with 1000 steps in the chain, 100 batches, and 1000 iterations per batch. We used the binomial likelihood approach for multiple tests (Chapman et al. 1999) to test for significant heterozygote deficits and departures from LE across all sites for each locus (heterozygotes) or pair of loci (linkage). This was done using the likelihood function in Chapman et al. (1999):

\[ L = \sum_{i=n}^{n} C(1 - \alpha)^{n-r}(\alpha)^r, \]

where \( n \) is the total number of tests, \( r \) is the number of significant tests, \( \alpha \) is the significance level (0.05), and \( C \) is a factorial constant \( \left( \frac{n!}{r!(n - r)!} \right) \). Values are summed from \( r \) to \( n \). If \( L \) is lower than \( \alpha \), we consider tests for departures from HWE and LE to be significant overall.

To detect changes in genetic variability, we examined allelic richness and heterozygosity. To estimate allelic richness (i.e., the number of alleles standardized to a common sample size), we used HP-Rare version 8-16-2004 (Kalinowski 2005), assuming 30 genes per site (as in Crispo and Chapman 2008). We estimated within-site observed (direct count) and expected (unbiased)
heterozygosities across all loci using Tools for Population Genetic Analysis version 1.3 (Miller 1997). Inbreeding coefficients ($F_{IS}$) were calculated at each site using the equation $F_{IS} = 1 - (H_o/H_e)$, where $H_o$ and $H_e$ are the observed and expected heterozygosity, respectively.

To examine whether effective population sizes ($N_e$) changed between years, we used the program LDNE (Waples and Do 2008). This program estimates $N_e$ using information on linkage disequilibrium (LD), based on point-in-time samples, and corrects for biases resulting from the presence of rare alleles. Because alleles with very low frequencies can bias results (Waples 2006), we performed analyses after removing alleles with frequencies ($F_{crit}$) lower than 0.05, 0.02, and 0.01 (i.e., 3 separate analyses). We used a model of random mating and used both the parametric and jackknife procedures to construct 95% confidence intervals. Similarly, we estimated the effective number of breeding individuals ($N_b$) using the program Nb_HetEx (Zhadanova and Pudovkin 2008). This analysis is based on the assumption that the excess of heterozygotes increases with a decreasing number of breeders. We used 1000 iterations for the bootstrap analysis. All of these analyses were performed on both the 2006 and 2008 data. Note, however, that these 2 methods assume that populations are closed (i.e., that no gene flow occurs among populations), which is probably not the case in the present system (see below). Therefore, the results from these analyses should be interpreted with caution.

To detect changes in genetic structure, we estimated $F_{ST}$ values for all site pairs within a year, and between years within each site, based on distance matrices using Arlequin version 3.01 (Excoffier et al. 2005). Significance levels of $F_{ST}$ values were based on 1000 permutations. Next, we performed 2 Mantel tests using Fstat version 2.9.3.2 (Goudet 2001). The first Mantel test compared matrices of within-year $F_{ST}$ values, considering only sites that were common between years (2008 values used as the dependent variable, 2006 values used as the explanatory variable). The second Mantel test tested for isolation by distance using the 2008 data set, comparing matrices of $F_{ST}$ values and geographical distance (results from the 2006 data set are presented in Crispo and Chapman 2008). Geographical distance separating sites was measured as the physical distance along the waterway. Significance levels for the Mantel tests were based on 20,000 randomizations.

To examine the relative effects of spatial versus temporal variation, we performed an analysis of molecular variance (AMOVA; locus-by-locus and over all loci) using Arlequin version 3.01, considering only the 5 sites that were common between years. Genetic variation was partitioned 3 ways: among sites across years (i.e., years nested within sites), among years within sites, and within sites within years. Significance levels for the AMOVA groupings were based on 1000 permutations. This analysis will inform whether structuring is significant among sites irrespective of time (i.e., spatial structure) versus among years within sites (i.e., temporal structure).

Genetic structure was too low to obtain consistent and meaningful results using the programs GENECLASS or MIGRATE—the former would have detected first-generation dispersers and the latter would have estimated directional gene flow between pairs of sites and between years within sites. We attempted to use both of these programs, but GENECLASS indicated that over 80% of sampled individuals were first-generation dispersers (accurate detection should be low when $F_{ST}$ is low; Manel et al. 2005), and MIGRATE provided inconsistent results even after very long runs (results not shown). In addition, MIGRATE assumes that population dynamics are at a stable equilibrium, which is not the case in the present system, making its use inappropriate for the current data set (see Kuhner 2009). Instead, we estimated the proportion of shared alleles ($D_{ps}$; Bowcock et al. 1994), which provides some information on population admixture (assuming that identical alleles are a result of common ancestry and are not due to homoplasy). We performed this analysis using Microsatellite Analyzer version 4.05 (Dieringer and Schlötterer 2003), where $D_{ps}$ was calculated using $1 - (similarity \ factor)$.

**Results**

We detected significant heterozygote deficits for one locus in the 2008 data set (Pmv9; $P = 0.0328$) after correcting for multiple tests. Using the binomial likelihood approach for multiple tests (Chapman et al. 1999), any locus that was found to have significant heterozygote deficits in 2 or more of the 6 sites was considered to have significant heterozygote deficits overall and thus possible null alleles. We also detected significant heterozygote deficits for this locus when testing across all sites simultaneously ($P = 0.0213 \pm 0.0102$ standard error). However, in the 2006 data set, heterozygote deficits were not detected for any of the currently used loci (Crispo and Chapman 2008). A possible reason for deviations from HWE in 2 sites (Rwebakwata and Kahunge) in the 2008 data set is the presence of first-generation dispersers. It remains curious, however, why only one locus would show reductions in heterozygosity. Because this locus was used in the 2006 analysis, we also used it in the present analysis so that between-year comparisons could be made, but we also estimated $F_{ST}$ values without Pmv9 and for Pmv9 only. We excluded Pmv9 to determine the robustness of the results, and we used Pmv9 only to observe whether this locus behaves differently than the other loci. We refer only to results obtained using all 10 loci unless otherwise specified.

We detected significant departures from LE for 3 pairs of loci in the 2008 data after correcting for multiple tests: Pmv4 and Pmv17 ($P < 0.0001$), Pmv3 and Pmv13 ($P = 0.0328$), and Pmv3 and Pmv17 ($P = 0.0328$). When LE was tested across all sites simultaneously, only 2 of these 3 pairs were in LD (Pmv4 and Pmv17; Pmv3 and Pmv13; $P < 0.001$ for both). The only pair of loci that showed LD in the 2006 data set was Pmv4 and Pmv17, but only when tested across sites simultaneously (Crispo and Chapman 2008). In the 2008 data set, however, we detected LD in all 6 sites for these loci. It thus remains dubious whether these loci are...
actually physically linked or whether observed patterns are due to the sampling of first-generation dispersers; that is, allele combinations common to one site might have been introduced into another site in which different allele combinations are common. Because both of these loci were used in the 2006 data set, we also used them in the present analysis so that between-year comparisons could be made.

Both allelic richness and observed and expected heterozygosities decreased in 2008 relative to 2006 (Table 1; Crispo and Chapman 2008). There were 2 exceptions, where values were higher in 2008: allelic richness for Kahunge and observed heterozygosity for Kamwenge (but “expected” heterozygosity was lower in 2008 for all sites). However, when considering loci individually, not all loci showed decreases in allelic richness and heterozygosity in 2008 (Table 1). Genetic diversity showed some directionality—the most downstream river site (Kamwenge) had the highest allelic richness and heterozygosity and the most upstream river site (Bunoga) had the lowest (Table 1). The pattern of increasing diversity in the downstream direction was more pronounced in the 2008 data set than in the 2006 data set (Table 1; Crispo and Chapman 2008). In the swamp, allelic richness and heterozygosity were higher in the site most distant from the river (Kanyantale; Table 1; Figure 2). This pattern for the swamp was also evident in the 2006 data set (Table 1; Crispo and Chapman 2008). In the swamp, decreases in allelic richness and heterozygosity in 2008 when considering loci individually, not all loci showed increases in allelic richness and heterozygosity from 2006 (Table 1; Crispo and Chapman 2008). In the swamp, values were higher in 2008: allelic richness for Kahunge and heterozygosities decreased in 2008 relative to 2006 (Table 1; Crispo and Chapman 2008). In the swamp, both allelic richness and observed and expected heterozygosities decreased in 2008 relative to 2006 (Table 1; Crispo and Chapman 2008). In the swamp, very large confidence intervals were obtained for estimates of Ne and Nb, often with upper bounds at infinity. Because of the high number of alleles (Table 1), and which alleles were excluded from the analysis (data not shown). Using the LD method, the estimated Ne tended to increase with decreasing Pcrit, the allele frequency below which alleles were excluded from the analysis (data not shown). Because of the high number of alleles (Table 1), and thus low allele frequencies, in our data sets, we present only results from the analyses using the lowest Pcrit, that is, 0.01 (Table 2). In addition, confidence intervals for Ne calculated using the jackknife procedure were always larger than those calculated using the parametric procedure (data not shown). Because confidence intervals for Ne estimates were large in general, we present only those calculated using the parametric procedure (Table 2). Overall, we observed no trends in Ne or Nb across methods of estimation (Table 2). The LD method revealed only a significant decrease in Ne between years for Bwera, whereas the heterozygote-excess method revealed only a significant decrease in Nb between years for Kamwenge and Kahunge (and only when using the parametric method; Table 2). A possible reason for these inconsistent results is that the methods used for estimation assume that populations are closed (i.e., no gene flow) and that cohorts are discreet (i.e., no overlapping generations) (Pudovkin et al. 1996; Waples and Do 2008). Neither of these assumptions is realistic in the present system. Another problem with estimating Ne is the difficulty in defining a population. Sampling sites probably do not reflect discreet populations—instead, populations are probably continuous along the stream and throughout the swamp.

Changes in genetic structure were documented between years. First, FST values tended to be lower in 2008, with only one pair of sites showing a significant FST value in 2008 but not in 2006 (Bwera and Rwembakwata; Table 3). Second, 2 FST values “between” years “within” sites were significant, and these values were higher than some within-year FST values (Kahunge, Kamwenge; Table 4). These results were similar when Pmv9 (i.e., the locus out of HWE) was excluded from the analyses (Tables 3 and 4). Third, Mantel tests did not detect a significant correlation in FST values between years (r2 = 0.2587; P = 0.1290). Fourth, isolation by distance was detected in 2006 (Crispo and Chapman 2008) but not in 2008 (r2 < 0.0001; P = 0.9944; Figure 3). Fifth, AMOVA detected significant partitioning of genetic variation between years when averaged over all loci and for 7 of the 10 loci when tested individually (Table 5). Sixth, although the proportion of shared alleles (Dps) was high between sites within years, there was a slight tendency for this proportion to be lower in 2008 (Table 3).

Pmv9 produced some results that differed from those produced using the other loci. In addition to the presence of heterozygote deficits for this locus (above), some patterns of FST differed (Tables 3 and 4). The most striking result was an

| Table 1. Microsatellite variability for sites sampled in 2008 (2006 values in brackets, from Crispo and Chapman 2008) |
|-----------------|-----------------|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Swamp           |                 |                               |                              |                                |                 |
| Kanyantale      | 34 (27)         | 136 (147)                     | 112.5 (3.1)                  | 0.9202 (0.9193)              | 0.8697 (0.8754) | −0.0580 (−0.0231) |
| Bwera           | 29 (27)         | 130 (147)                     | 104.4 (3.4)                  | 0.8605 (0.913)              | 0.8411 (0.8754) | −0.0265 (−0.0502) |
| River           |                 |                               |                              |                                |                 |
| Bunoga          | 30 (31)         | 136 (150)                     | 109.0 (5.4)                  | 0.8249 (0.8742)             | 0.8337 (0.8415) | 0.0106 (−0.0389)  |
| Rwembakwata     | 30 (31)         | 148 (144)                     | 115.0 (5.3)                  | 0.8778 (0.9026)             | 0.8602 (0.8794) | 0.0205 (−0.0264)  |
| Kahunge         | 30 (30)         | 152 (144)                     | 119.6 (7.2)                  | 0.8906 (0.8917)             | 0.8587 (0.8687) | 0.0365 (−0.0265)  |
| Kamwenge        | 30 (18)         | 165 (143)                     | 126.1 (15.9)                 | 0.9185 (0.8874)             | 0.8795 (0.8879) | −0.0443 (0.0006)  |

n is the sample size, Hc is observed heterozygosity, Hc is expected heterozygosity, Fis is 1 − (Hc/Hc). The italicized values are the number of loci showing higher values in 2006 than in 2008.
increase in \( F_{ST} \) between years for the Bwera site (Table 4)—a significant \( F_{ST} \) value for this site was obtained using Pmv9 but not using all loci combined. In addition, in the AMOVA, Pmv9 was the locus with the lowest percentage of variation among sites and had a high percentage of variation between years (Table 5). We predict that this locus might be weakly linked to a locus under selection between years, perhaps most strongly influenced by selection in the swamp.

**Discussion**

Our results show changes in genetic variability and genetic structure on a time scale of 2 years or approximately 4 generations. Most striking, strong isolation by distance had been detected in 2006 (Crispo and Chapman 2008) but was not evident in 2008 (Figure 3). We can envision 4 possible ways in which these genetic changes could have accrued: 1) fluctuations in population sizes, 2) increased gene flow among sites, 3) altered patterns of natural selection, and/or 4) genetic drift. We will discuss each of these possibilities in turn and how they are supported by our results.

**Population Sizes**

We observed slightly lower allelic richness and heterozygosities in 2008 than in 2006, although this pattern was not consistent among loci at all sites (Table 1). Lower genetic diversity has been linked to lower effective population sizes (Crow and Kimura 1970) and thus these results provide evidence that effective sizes might have declined between 2006 and 2008. This decline could be due to changes in abiotic or biotic conditions between years (see below) or through the physical movement of fish (e.g., Fausch et al. 2001; Grether et al. 2001; Barson et al. 2009). Our estimates of \( N_e \) and \( N_b \), however, did not reveal any general trends in changes in population sizes between years (Table 2). As noted above, a possible reason for the high variation in these estimates is that gene flow is high among sampled sites, violating the assumptions of the tests that we used.

**Gene Flow**

Some evidence suggests that increased directional gene flow might have occurred between years. In the river, there was a slight tendency for genetic diversity to increase in the downstream direction, and this pattern was more pronounced in the 2008 data set than in the 2006 data set, suggesting higher than usual downstream gene flow between sampling years (Table 1; Crispo and Chapman 2008). In the swamp, allelic richness and heterozygosities were higher in the site most distant from the river (Kanyantale; Table 1; Figure 2). Because \( P. \) multicolor could not be caught in abundance at sites that were explored upstream (or "upsamp") of our sampling sites (Aliganyira E, Omeja P, Twinomugisha D, personal communications; Chapman LJ, personal observations), it is not likely that significant gene flow is occurring from upstream nonsampled sites—it is more probable that
gene flow occurs from the river up through the swamp, which is plausible because water flow in the swamp is low. This pattern for the swamp was also evident in the 2006 data set, suggesting that gene flow is generally higher in this direction within the swamp (Table 1; Crispo and Chapman 2008).

Some other results suggest increased downstream gene flow between years. First, the 2 $F_{ST}$ values that were higher in 2008 were for site pairs located upstream (Bunoga vs. Bwera and Bwera vs. Rwebakwata); that is, $F_{ST}$ was lower for downstream sites (Kahunge and Kamwenge; Table 3; Figure 2). As well, the 2 site-pairs for which $F_{ST}$ values were significant in 2006 but not in 2008 (Bunoga vs. Kahunge and Rwebakwata vs. Kamwenge) each contain one site located downstream (Table 3; Figure 2). Second, the 2 sites that had significant $F_{ST}$ values between years within sites were the sites located farthest downstream in the river (Kahunge and Kamwenge) (Table 4; Figure 2).

We observed that the proportion of shared alleles among sites decreased in 2008 relative to 2006, and this decrease could be due to slight decreases in allelic richness in 2008 relative to 2006. Low private allelic richness in both years (Table 1) and a high proportion of shared alleles among sites (Table 3) indicate that gene flow is high overall. Even though the proportion of shared alleles was high “among” sites, there was a tendency for it to be even higher between years within sites (Table 4). Similarly, the AMOVA revealed a greater proportion of variation among sites than between years.

### Natural Selection

A possible reason for variation in neutral genetic diversity between years is that patterns of natural selection had...
neutral markers, small fluctuations in allele frequencies can
in systems characterized by low genetic differentiation at
Genetic Drift
enced different patterns of selection during development.
of a mixture of cohorts, each of which might have experi-
sampled individuals, however, and samples probably consist
of selection might have acted on juveniles from different
overall (Palm et al. 2003), suggesting that different patterns
among sites during periods of nonflooding. We estimate that
anywhere from a fraction of a year to nearly 19 years would be
required for the build-up of genetic differences among sites similar to those observed in 2006. We assume 2 generations per year and use 100, 500, and 1000 as values of \( N \). Implicit in this equation is the assumption that no gene flow occurs among sites during periods of nonflooding. We estimate that anywhere from a fraction of a year to nearly 19 years would be required for the build-up of \( F_{ST} \) between sites for which \( F_{ST} \) was lower in 2008 (Table 6), depending on the estimated value of \( N \) and the site pair considered. Notably, the time required generally increases with geographical distance between sites, reflecting a pattern of isolation by distance (Table 6). It is therefore likely that gene flow occurs continuously in this system, with higher gene flow between sites that are geographically close to each other. Therefore, the above equation might not be valid for this system, and these estimates should be interpreted with caution.

Effects of Floods?
Genetic structure can be strongly influenced by flooding in riverine systems (e.g., Congdon 1995; Jowers et al. 2008; Masci et al. 2008; Barson et al. 2009). Because patterns of rainfall in the study area remained relatively constant over a number of years (Figure 1), such striking changes in genetic structure are surprising. However, rainfall measures might not directly correlate with water flow, and thus undetected differences in water flow among seasons might have influenced fish population structure between years. Indeed, over a 2-year period, we noted a nonsignificant relationship between monthly water depth readings at Kahunge (Figure 2) and monthly rainfall at this site and at locations upstream of this site (data not shown). We noted higher water levels during the second sampling season than the first (Crispo E, personal observations), even though both sets of samples were collected in May–June, indicating that water discharge might have increased between years. The effects of water flow could influence population structure directly, or population structure could be influenced by other biotic or abiotic properties that affect fish movement or survival (e.g., Congdon 1995; Fauch et al. 2001; Grether et al. 2001).

If the observed genetic patterns in 2008 were, in fact, influenced by a flooding event that occurred between 2006 and 2008, how much evolutionary time would be required for the build-up of genetic differences among sites similar to those observed before the flood? That is, given the \( F_{ST} \) values observed in 2008, how much time is needed until genetic differences among sites are similar to those observed in 2006? The answer to this question would shed light on how often large floods, or similar events, occur in this system to produce genetic change. We consider the following equation for the relationship between \( F_{ST} \) at 2 points in time (Hartl 2000, p. 65):

\[
1 - F_{ST} = (1 - F'_{ST})(1 - (1/(2N)))^t,
\]

where \( F_{ST} \) is the value before the flood (2006), \( F'_{ST} \) is the value after the flood (2008), \( N \) is the population size, and \( t \) is the number of generations between \( F'_{ST} \) and \( F_{ST} \). We use this equation to estimate the number of years since the last flooding event, prior to 2006, which resulted in \( F_{ST} \) values similar to those observed in 2008. We assume 2 generations per year and use 100, 500, and 1000 as values of \( N \). Implicit in this equation is the assumption that no gene flow occurs among sites during periods of nonflooding. We estimate that anywhere from a fraction of a year to nearly 19 years would be required for the build-up of \( F_{ST} \) between sites for which \( F_{ST} \) was lower in 2008 (Table 6), depending on the estimated value of \( N \) and the site pair considered. Notably, the time required generally increases with geographical distance between sites, reflecting a pattern of isolation by distance (Table 6). It is therefore likely that gene flow occurs continuously in this system, with higher gene flow between sites that are geographically close to each other. Therefore, the above equation might not be valid for this system, and these estimates should be interpreted with caution.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Among sites</th>
<th>Between years within sites</th>
<th>Within sites within years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmv1</td>
<td>0.6758</td>
<td>−0.1112</td>
<td>99.4354</td>
</tr>
<tr>
<td>Pmv5</td>
<td>0.5892</td>
<td>0.3753</td>
<td>99.0355</td>
</tr>
<tr>
<td>Pmv4</td>
<td>0.4537</td>
<td>0.3311</td>
<td>99.2152</td>
</tr>
<tr>
<td>Pmv9</td>
<td>0.0872</td>
<td>0.6478</td>
<td>99.2650</td>
</tr>
<tr>
<td>Pmv13</td>
<td>0.3492</td>
<td>0.0155</td>
<td>99.6353</td>
</tr>
<tr>
<td>Pmv15</td>
<td>0.8086</td>
<td>−0.4753</td>
<td>99.6667</td>
</tr>
<tr>
<td>Pmv17</td>
<td>0.4389</td>
<td>0.4233</td>
<td>99.1377</td>
</tr>
<tr>
<td>Ppun4</td>
<td>1.4324</td>
<td>−0.8953</td>
<td>99.4628</td>
</tr>
<tr>
<td>Ppun5</td>
<td>0.3393</td>
<td>0.4989</td>
<td>99.1618</td>
</tr>
<tr>
<td>Ppun17</td>
<td>0.4886</td>
<td>0.8032</td>
<td>98.7082</td>
</tr>
<tr>
<td>All</td>
<td>0.5241</td>
<td>0.2148</td>
<td>99.2611</td>
</tr>
</tbody>
</table>

Values for all loci are based on the weighted averages. Bold values indicate significant (\( \alpha = 0.05 \)) variance components (1000 permutations).

Genetic Drift
In systems characterized by low genetic differentiation at neutral markers, small fluctuations in allele frequencies can greatly influence the accuracy and precision of \( F_{ST} \) estimates (Waples 1998). The \( F_{ST} \) values estimated in our study are much lower than the average estimates for freshwater fishes (Table 3; Ward et al. 1994; Waples 1998), indicating very low genetic differentiation in this system. Small demographic changes from one year to the next, or slight deviations from random sampling, could have influenced the results.

Table 5. Percentage of variation in each grouping obtained from AMOVA

Journal of Heredity 2010:101(1)
Table 6. Time required (in years) for $F_{ST}$ values after a putative flood (2008 values) to build up to values observed in 2006.

<table>
<thead>
<tr>
<th>Site 1</th>
<th>Site 2</th>
<th>N = 100</th>
<th>N = 500</th>
<th>N = 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunoga</td>
<td>Rwebakwata</td>
<td>0.14</td>
<td>0.70</td>
<td>1.41</td>
</tr>
<tr>
<td>Bunoga</td>
<td>Kahunge</td>
<td>0.47</td>
<td>2.36</td>
<td>4.73</td>
</tr>
<tr>
<td>Bunoga</td>
<td>Kamwenge</td>
<td>1.87</td>
<td>9.37</td>
<td>18.74</td>
</tr>
<tr>
<td>Bwera</td>
<td>Kamwenge</td>
<td>0.61</td>
<td>3.08</td>
<td>6.15</td>
</tr>
<tr>
<td>Rwebakwata</td>
<td>Kamwenge</td>
<td>1.10</td>
<td>5.49</td>
<td>10.99</td>
</tr>
<tr>
<td>Kahunge</td>
<td>Kamwenge</td>
<td>1.88</td>
<td>9.42</td>
<td>18.84</td>
</tr>
</tbody>
</table>

Assumes 2 generations per year.

Conclusions

Our results unequivocally show that changes in genetic structure accrued between years in this system. However, it is difficult to ascertain the causes of these changes. Our results provide some evidence that changes are due to increased gene flow and possibly changes in population sizes and patterns of natural selection. Because genetic structure is low overall, small fluctuations in allele frequencies, such as those due to drift or nonrandom sampling, could have had large impacts on the results. The most probable explanation is that a combination of these factors influenced genetic change in this system between years.

In conclusion, population genetic structure of riverine fishes can fluctuate over short time scales. Similar results have been found in some other freshwater systems (e.g., Congdon 1995; Beneteau et al. 2009), but they conflict with results from some other studies of aquatic systems, which showed genetic structure to be stable across years (e.g., Favé and Turgeon 2008; Gonzalez et al. 2008; Ungfors et al. 2009). More studies are needed to determine under what scenarios temporal variation is likely to occur. Systems characterized by low differentiation might be particularly prone to show variation in genetic patterns over time, which can be greatly influenced by slight variation in demographic parameters. Estimates based on samples taken at one point in time might thus not be valid across time. Understanding how population structure is expected to fluctuate on short time scales is imperative for knowing how populations will respond over longer periods of time and for establishing conservation policies. Future work on riverine organisms should examine temporal changes in genetic structure, rather than drawing conclusions based on point-in-time estimates.

Funding

Natural Sciences and Engineering Research Council of Canada in the form of a Canada Graduate Scholarship (to E.C.), a Discovery Grant (to L.J.C.), and Canada Research Chair funds (to L.J.C.).

Acknowledgments

Field work was completed with the help of Emmanuel Aliganyira, Patrick Omeja, Jaelyn Paterson, Diana Sharpe, and Dennis Twinomugisha. Genotyping was completed by Genevieve Geneau at Génomique Québec Innovation Centre. The rainfall data for Kahunge were provided by Erin Reardon. Helpful comments were provided by Alison Derry, Marie-Julie Favé, Andrew Hendry, Adam Herman, Simon Joly, Daniel Schoen, Robin Waples, and 2 anonymous reviewers.

References

Chapman LJ. 1995. Seasonal dynamics of habitat use by an air-breathing catfish (Carassius surinamensis) in a papyrus swamp. Ecol Fresh Fish. 4:113–123.


Received March 17, 2009; Revised July 26, 2009; Accepted August 12, 2009

Corresponding Editor: Robin Waples